

Determinants of fine-scale homogenization and differentiation of native freshwater fish faunas in a Mediterranean Basin: implications for conservation

Virgilio Hermoso^{1*}, Miguel Clavero^{2,3} and Mark J. Kennard¹

¹Australian Rivers Institute, Griffith University, Nathan, Queensland 4111, Australia, ²Estación Biológica de Doñana - CSIC, Americo Vespucio s/n, 41092 Sevilla, Spain, ³Grup d'Ecologia del Paisatge, Àrea de Biodiversitat, Centre Tecnològic Forestal de Catalunya, Carretera vella de Sant Llorenç de Morunys km. 2, 25280 Solsona

ABSTRACT

Aim Increasing threats to freshwater biodiversity are rapidly changing the distinctiveness of regional species pools and local assemblages. Biotic homogenization/differentiation processes are threatening the integrity and persistence of native biodiversity patterns at a range of spatial scales and pose a challenge for effective conservation planning. Here, we evaluate the extent and determinants of fine-scale alteration in native freshwater fish assemblages among stream reaches throughout a large river basin and consider the implications of these changes for the long-term conservation of native fishes.

Location Guadiana River basin (South-Western Iberian Peninsula).

Methods We quantified the magnitude of change in compositional similarity between observed and reference assemblages and its potential effect on natural patterns of compositional distinctiveness. Reference assemblages were defined as the native species expected to occur naturally (in absence of anthropogenic alterations) and were reconstructed using a multivariate adaptive regression splines predictive model. We also evaluated the role of habitat degradation and introduced species as determinants of biotic homogenization/differentiation.

Results We found a significant trend towards homogenization for native fish assemblages. Changes in native fish distributions led to the loss of distinctiveness patterns along natural environmental gradients. Introduced species were the most important factor explaining the homogenization process. Homogenization of native assemblages was stronger in areas close to reservoirs and in lowland reaches where introduced species were more abundant.

Main conclusions The implementation of efficient conservation for the maintenance of native fish diversity is seriously threatened by the homogenization processes. The identification of priority areas for conservation is hindered by the fact that the most diverse communities are vanishing, which would require the selection of broader areas to adequately protect all the species. Given the principal role that introduced species play in the homogenization process and their relation with reservoirs, special attention must be paid to mitigating or preventing these threats.

Keywords

Biological invasions, fish conservation, habitat degradation, introduced species, reference condition.

*Correspondence: Virgilio Hermoso, Australian Rivers Institute, Griffith University, Nathan, Queensland 4111, Australia.
E-mail: virgilio.hermoso@gmail.com

INTRODUCTION

Human activities are increasingly threatening freshwater biodiversity and altering natural patterns of biotic distinctiveness (Olden *et al.*, 2010; Strayer & Dudgeon, 2010). The combination of species introductions and extinctions is causing changes to spatial patterns of compositional similarity at a range of scales (Olden *et al.*, 2004; Rahel, 2010). These changes translate into homogenization or differentiation of biodiversity. Taxonomic homogenization is defined as an increase in the similarity of the pools of species found among a set of assemblages over time (McKinney & Lockwood, 1999; Olden *et al.*, 2004). This process is rapidly leading to a global diminution of distinctiveness in terrestrial and aquatic biotas and causing the reorganization of natural patterns in species richness and their spatial turnover (Marchetti *et al.*, 2006). On the other hand, taxonomic differentiation (i.e. decrease in compositional similarity) can occur as a result of the idiosyncratic nature of species introductions. The extent of homogenization/differentiation observed can also depend on the spatial scale of examination and the temporal stage of the faunal alteration process (Clavero & Garcia-Berthou, 2006; Olden *et al.*, 2008; Rahel, 2010).

Freshwater fish are the biological group most frequently addressed in studies of taxonomic homogenization. Changes in similarity of fish faunas have been examined at different spatial scales across different continents (see Rahel, 2010, Marr *et al.*, 2010). Overall, the results of such studies report a consistent pattern towards increasing homogenization (Marchetti *et al.*, 2001; Rahel, 2002, 2010; Olden *et al.*, 2008). To date, researchers have made little progress in elucidating the key ecological and environmental determinants and consequences of biotic homogenization (Olden & Rooney, 2006; Olden *et al.*, 2010). Environmental change (e.g. through habitat degradation) and species introductions promote the geographical expansion of some species and the reduction in others, ultimately leading to biotic homogenization (e.g. Walters *et al.*, 2003; Taylor, 2004; Marchetti *et al.*, 2006; Olden *et al.*, 2008). Introduced fishes are recognized as one of the major causes of the worldwide decline of aquatic fauna (Cowx, 1998; Clavero & Garcia-Berthou, 2005; Helfman, 2007), and their spread is generally associated with loss of native biodiversity (Collares-Pereira *et al.*, 2002; Clavero *et al.*, 2004; Hermoso *et al.*, 2011). In addition, native fish species loss and faunal homogenization have been attributed to deterioration of habitat quality associated with urbanization, changes in land uses and sedimentation (Walters *et al.*, 2003; Marchetti *et al.*, 2006; Smart *et al.*, 2006), and more predominantly to river damming (Rahel, 2002; Gido *et al.*, 2004, 2009).

Determining the relative role of different threats in the biodiversity alteration process is an essential step for the development and implementation of efficient and effective management actions to minimize or reverse these threats (Didham *et al.*, 2005). The spatial co-occurrence of different threats makes it difficult to discriminate the relative contribution of each perturbation factor to the change in native communities (Kennard *et al.*, 2005; Light & Marchetti, 2006;

Clavero *et al.*, 2010; Hermoso *et al.*, 2011). The consideration of homogenization/differentiation processes is of special concern for conservation since changes in patterns in species turnover can be harbingers of conservation failures (Whittaker *et al.*, 2005). Homogenization will make conservation practitioners struggle in the already difficult task of finding the best combination of places that maximize the representation of regional biodiversity while minimizing economic, political or social costs of effective management actions. This will be in detriment to the success of conservation practice. In a recent paper on biotic homogenization and conservation prioritization, Rooney *et al.* (2007) posed an important question on how can we best evaluate biotic homogenization, so it reflects changes in biota that are relevant for conservation purposes. They pointed out that quantitative studies of biotic homogenization can provide useful insights into conservation problems when used appropriately but can be dangerously misleading when they are not. For example, biotic differentiation can be led by the loss of native species, while biotic homogenization can be hidden by the substitution of native species by new introduced species. It is for this reason that the study of biotic homogenization with a conservation aim must be cautiously taken. By separating the concept of biotic homogenization at the global scale from the study of biotic homogenization at spatially- and temporally explicit scales, researchers can avoid many of the subtle pitfalls inherent in homogenization studies (Rooney *et al.*, 2007).

Here, we aim to address two of the main challenges highlighted in previous reviews on conservation biogeography, such as how to best analyse homogenization/differentiation with a conservation aim (Rooney *et al.*, 2007) and the need to discern the role of the different determinants of such processes (Olden, 2006). We quantify the extent of changes in compositional similarity of freshwater fish faunas among stream reaches of a Mediterranean Basin (Guadiana River) and whether this has led to homogenization or differentiation processes. We also explore whether these potential changes have altered natural patterns in compositional similarity. To guide future conservation management, we analyse the role of different determinants, such as habitat degradation and introduced species, in this process. We address Rooney *et al.* (2007) question by evaluating fish faunal alteration at the reach scale within a single river basin as this is the basic management unit in freshwater conservation planning (Abell *et al.*, 2007). In addition, to avoid the effect of introduced species in the estimates of homogenization/differentiation rates, we focus our attention on native species only. To our knowledge, this is the first attempt to study changes of compositional similarity of native freshwater fish at this fine scale across a whole catchment.

METHODS

Study area

The Guadiana River basin is located in the South-Western Iberian Peninsula draining a total area of 67,039 km² to the

Atlantic Ocean (Fig. 1). It features a typical mediterranean climate, with high intra- and interannual variation in discharge and severe floods and droughts (Gasith & Resh, 1999).

Nearly half of the basin (49.1%) is currently being used for agriculture with about 25% farmed intensively. As a result of the large agricultural water demand, about 13,000 GL of water is stored in 88 large (>1 GL) and more than 200 smaller (<1 GL) reservoirs causing major alterations to natural surface flow regimes. Substantial groundwater extraction occurs in some areas of the basin, from thousands of legal wells and an unknown number of illegal ones, with potentially severe consequences for the main aquifers. Other common human perturbations include direct modification of river channels, the degradation of the riparian forests and the deterioration of water quality (Hermoso *et al.*, 2010).

Fish community and habitat characterization

Fish communities were characterized in 239 localities across the basin (Fig. 1) during spring in 2002, 2005 and 2006. Fish sampling at each location was carried out along 100-m-long stream reaches whenever possible using single-pass electro-fishing without block nets. The sampling stretch covered all habitats available following the recommendations of the FAME Consortium (2004) for European rivers. Sály *et al.* (2009) and Bertrand *et al.* (2006) demonstrated this method to be adequate to characterize spatial and temporal trends in fish abundance and composition. Fish abundances were standardized as catch per unit of effort by the total length of stream surveyed and time devoted [CPUE = number of fish/(stream length (m) × time (hours))]. Rare species (<5% prevalence) were deleted from the analyses, because they are more likely to occur randomly in samples and may not represent true

differences in assemblage structure across space or time (Falke & Gido, 2006). Only sites with at least one native species were considered for further analyses ($n = 156$).

Habitat was characterized by 59 environmental variables, covering three different spatial scales: site, reach (buffer area of 500 m around the sampling point) and basin (upstream area draining to the sampling point). They included (1) *in situ* measures, which described micro- and mesohabitat characteristics at each sampling location and (2) remotely collected data, using geographical information systems (GIS; Table S1). All variables were checked for normality and transformed when necessary prior to analysis (arcsine for land-cover variables – expressed as proportion – and $\log(x + 1)$ for the remaining variables).

We performed two principal components analyses (PCA) on the variables listed in Table S1 to summarize the major natural and anthropogenic environmental gradients in the basin. The first PCA was carried out on environmental variables not affected by human perturbation (Table 1), so the gradient extracted reflected natural changes. The first two principal components (PCs) retained the 44.4% of the original variance and mainly described a climatic gradient (PC_{1nat}) of decreasing temperature, decreasing seasonality of precipitation, increasing temperature seasonality and increasing elevation and a river size gradient (PC_{2nat}) of increasing river width, distance to headwater and catchment area (Table 1). The second PCA was performed on variables describing human impairment to obtain gradients of perturbation. Distances to reservoirs were not included in this second PCA to use them as independent variables in further analyses. The first two PCs explained the 44.7% of the original variance and were related to the degree of land transformation at the basin and reach scale and perturbation of the riparian forest (PC_{1deg}), and a water quality and

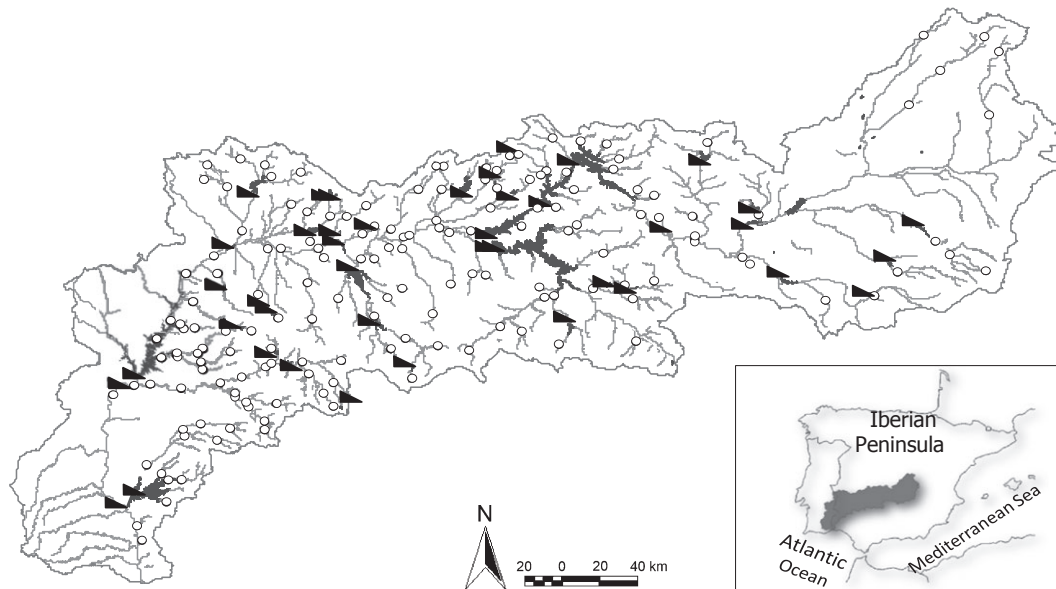


Figure 1 Location of 239 sampling sites in the Gadiana River basin (indicated with white circles). The spatial distribution of dams is indicated with black triangles, and the extent of reservoirs is shown in black shading.

Table 1 Principal component analysis (PCA) used to summarize the set of environmental variables included in Table 1. Only variables not affected by human perturbations were included in PC_{nat}, while PC_{pert} summarizes perturbation variables. Only variables with a score >|0.5| are shown here. R denotes variables measured at the reach scale, while B indicates basin scale variables (Table 1).

Variable	PC1 _{nat} (29.6%)	PC2 _{nat} (14.8%)
Coldest temperature (R)	-0.92	
Mean temperature (B)	-0.91	
Mean temperature (R)	-0.90	
Precipitation seasonality (R)	-0.81	
Precipitation seasonality (B)	-0.78	
Temperature seasonality (B)	0.71	
Temperature seasonality (R)	0.71	
Maximum elevation (B)	0.82	
Maximum elevation (R)	0.92	
Mean elevation (B)	0.94	
Relative position		0.77
River width		0.84
Distance to headwater		0.93
Basin area		0.93
	PC1 _{pert} (29.1%)	PC2 _{pert} (15.6%)
Intensive agriculture (B)	0.92	
Intensive agriculture (R)	0.87	
QBR	-0.51	
Natural (R)	-0.85	
Natural (B)	-0.91	
PO ₅ ³⁻ (mg L ⁻¹)		0.89
NH ₄ ⁺ (mg L ⁻¹)		0.81
Human footprint (B)		0.61
Cl ⁻ (mg L ⁻¹)		0.61
Population density (R)		0.56

human density gradient (PC2_{deg}) (Table 1). Potential covariation issues between PC_{nat} and PC_{deg} were ruled out for further analyses (Pearson's $R^2 < 0.1$ in all cases).

Reference assemblages

To quantify the potential homogenization/differentiation that native fish assemblages might have experienced, we compared observed assemblages against those expected to occur in the absence of major perturbations (reference scenario hereafter, *sensu* Reynoldson *et al.*, 1997). The original database was split into two subsets: non-perturbed ($n = 65$) and perturbed ($n = 91$) localities. Non-perturbed sites were defined following the approach proposed by Hermoso *et al.* (2009b). They were characterized by minimal degradation due to urban or agricultural land-use upstream or adjacent to each site. Furthermore, bank and channel structure and the riparian zone were in natural condition (see Hermoso *et al.*, 2009b; for more details). To ensure that freshwater fish communities at non-perturbed sites were not seriously impacted by introduced

fish, all sites where non-native species accounted for more than 5% of total fish abundance were also discarded from the non-perturbed data set (Kennard *et al.*, 2006).

The probability of occurrence of 10 native species under the reference scenario was modelled using multivariate adaptive regression splines (MARS, Leathwick *et al.*, 2005) on the observed presence-absence at non-perturbed localities and using only predictor variables describing natural environmental gradients (i.e. not influenced by human activities; see Table S1). MARS is a method of flexible nonparametric regression modelling (Elith & Leathwick, 2007). It is useful for modelling complex nonlinear relationships between response and explanatory variables with similar levels of complexity to that of a generalized additive model (GAM; Hastie, 1991). Model performance was evaluated using two complementary approaches: deviance explained and the area under the receiver operating characteristic (ROC) curve (Fielding & Bell, 1997). The area under the ROC curve (AUC) was assessed through a k-fold cross-validation procedure (Hastie *et al.*, 2001). In this process, the data set is randomly divided into 10 exclusive subsets and model performance is calculated by successively removing each subset, re-fitting the model with the remaining data and predicting the omitted data. The average error when predicting occurrence in new sites can then be calculated by averaging the AUC across each of the subsets (Leathwick *et al.*, 2005). An AUC > 0.6 is usually defined as an acceptable model performance (Fielding & Bell, 1997). Deviance complements AUC because it expresses the magnitude of the deviations of the fitted values from the observations. The MARS model fitted on the non-perturbed data set was used to predict the probability of occurrence of native species at all sites, so two different assemblages were available for each sampling site: observed and reference composition (modelled).

Probabilities of occurrence were transformed into presence-absence data for posterior analyses using the optimal threshold obtained from the *cost* method in the *presence-absence* package in R. This objective method finds the optimal threshold for each species that balances the relative cost of false positive and false negative predictions (Fielding & Bell, 1997).

Quantifying the extent of biotic homogenization and differentiation

We quantified the magnitude of alteration of native freshwater fish assemblages of the Guadiana River basin at the reach scale by measuring the change in compositional similarity (CS) between the reference and observed scenarios. We used the difference in the Bray-Curtis similarity index (Legendre & Legendre, 1998) of each pair of sites under the observed and reference scenarios ($\Delta CS = CS_{\text{observed}} - CS_{\text{reference}}$) as an indication of the extent of alteration (homogenization or differentiation). A positive ΔCS value indicates higher present pairwise compositional similarities than under reference conditions and hence homogenization, while negative values indicate differentiation (Olden & Rooney, 2006). To test the

null hypothesis of no significant change in compositional similarity between the reference and the observed scenarios, we used the test for homogeneity of multivariate dispersions (Anderson, 2006; Olden *et al.*, 2008). Multivariate dispersion is an estimate of beta-diversity based on the average dissimilarity from individual observation units to their group centroid in the multivariate space defined using a dissimilarity measure (Bray–Curtis in our case) (Anderson *et al.*, 2006). This test was used to account for the lack of independence of individual dissimilarities between pair of sites that would bias the estimate of P values (Anderson *et al.*, 2006). The observation units corresponded to sampling sites in our study, and groups were defined as reference or observed data. The test calculates an F statistic to compare the average distance of each sampling site to their centroid across groups. Statistical significance was estimated by permuting least-squares residuals 999 times. If native communities had not followed a significant homogenization process, we would expect the average multivariate dispersion values in the reference and observed scenarios not to differ significantly.

Quantifying natural patterns in compositional dissimilarity

To test the null hypothesis of not significant differences in compositional dissimilarity across the Guadiana River basin, we measured the average compositional dissimilarity (Bray–Curtis) across groups of sites defined on the natural environmental gradients. We split PC1_{nat} and PC2_{nat} into five groups containing a similar number of sites ($n = 31$ for groups 1–4 and $n = 32$ for group 5) and ran independent tests on the reference and observed data sets. To address the lack of independence in the comparison of dissimilarity values across groups, we applied the test of homogeneity of multivariate dispersion as explained above but using the environmental gradients as a grouping factor. The analyses on the reference data set will help to find out whether freshwater fish faunas in the Guadiana River basin showed any significant natural rate of change in compositional dissimilarity along natural gradients or were naturally homogeneous. If natural patterns in compositional dissimilarity existed and changes in fish assemblages over time had altered them, we would expect to find different results in the reference and observed analyses.

Quantifying potential determinants of biotic homogenization and differentiation

To evaluate the relative roles of multiple potential determinants of compositional similarity change over time and their interactions in a single analysis, we used structural equation modelling (SEM; Bollen, 1989). We tested the role of the distance to the nearest reservoir (log transformed), the abundance of introduced species [$\log(\text{CPUE}+1)$] and the first two PCs of the perturbation analysis as determinants of the homogenization/differentiation process in the SEM analyses. We also incorporated the natural gradients (PC1_{nat} and

PC2_{nat}) to study the effects of the other determinant factors in the environmental context where they occur. As an indicator of homogenization or differentiation for each sampling point, we used the difference in compositional similarity of each site between the observed and reference scenarios. To avoid the lack of independence owing to pseudoreplication, instead of using all the pairwise comparisons, we used a single composite estimate of change in compositional dissimilarity for each site. This composite value (D_i) was a weighted average of all the pairwise comparisons for each site (equation 1). Pairwise comparisons were weighted by the standardized environmental distance between each pair of sites. In this way, environmentally similar sites contributed more to the compositional similarity value than those that were environmentally dissimilar. Pairwise environmental distances were estimated as Bray–Curtis similarity from an environmental matrix that included the same variables as in PCA_{nat} analyses.

$$D_i = \frac{\sum_{j=1}^k S_{ij} \times d_{ij}}{k} \quad (1)$$

where S_{ij} is the difference in compositional similarity (observed – reference) and d_{ij} is the standardized environmental distance between sites i and j , respectively, and k is the number of pairwise comparisons for site i . SEM is a statistical technique used to analyse the interacting effects of different determinants of ecological change, being a useful technique in situations where the direct and indirect linkages among factors might be too complex to test experimentally (Wootton, 1994; Didham *et al.*, 2005). Within SEM, interaction hypotheses are translated into a series of regression equations that can be solved simultaneously to generate estimated covariance matrices. Then, each estimated matrix is evaluated against the observed covariance matrix by means of a goodness-of-fit index to determine whether the hypothesized model is an acceptable representation of the data.

We tested all the potential SEM models combining our set of candidate determinants. These models included a complete model where all the determinants and their interactions were introduced (Fig. 4), a simple model with only natural gradients as independent variables and all the intermediate combinations of determinants. We used two measures to estimate the importance of each determinant explaining our data. First, we used the chi-square and P values from a likelihood ratio test. This test measures the probability that the observed and expected covariance matrices differ by more than would be expected because of random sampling errors (Mitchell, 1993; Shipley, 2000). If the data are consistent with the model specified, no significant differences between the observed and expected covariance matrices are expected. However, the likelihood ratio test cannot be used to compare different models, since it fails to compensate for model complexity. This would inevitably lead to rank highly the most complex models, even when much simpler models fit the data nearly as well. For this reason, we also ranked the models using the Akaike information criterion (AIC), which penalizes for model

Table 2 MARS-GLM model performance. Deviance explained (%) indicates the reduction in deviance for each species with respect a null model. The discriminatory power of the model for each species is given through the AUC of the receiver operating characteristic (ROC) curve (calculated by k-fold re-sampling with its SD in parenthesis). The probability of occurrence of the remaining three species found in the Guadiana River basin (*Anguilla anguilla*, *Luciobarbus guiraonis* and *Alosa alosa*) could not be modelled due to their extremely low prevalence (two, one and one occurrences, respectively).

Species	Deviance explained (%)	ROC
<i>Anaocypris hispanica</i> (Steindachner, 1866)	0.67	0.78 (0.03)
<i>Luciobarbus comizo</i> (Steindachner, 1864)	0.55	0.85 (0.05)
<i>Luciobarbus microcephalus</i> (Almaça, 1967)	0.51	0.88 (0.03)
<i>Luciobarbus sclateri</i> (Günter, 1868)	0.30	0.80 (0.07)
<i>Iberochondrostoma lemmingii</i> (Steindachner, 1866)	0.14	0.63 (0.06)
<i>Pseudochondrostoma willkommii</i> (Steindachner, 1866)	0.40	0.73 (0.06)
<i>Cobitis paludica</i> (Buen, 1930)	0.23	0.70 (0.06)
<i>Salaria fluviatilis</i> (Asso, 1801)	0.71	0.87 (0.07)
<i>Iberocypris alburnoides</i> (Steindachner, 1866)	0.23	0.75 (0.03)
<i>Squalius pyrenaicus</i> (Günter, 1868)	0.25	0.69 (0.04)
Average	0.40	0.77 (0.05)

complexity. Lower AIC values indicate better fit (Burnham & Anderson, 2002). We expect models that did not include important determinants not to fit our data (significant chi-square test) and be low ranked by AIC.

All the statistical analyses were run in the free statistical software R, Version 2.1.1 (R Development Core Team., 2004).

RESULTS

The predictive model of fish species occurrences exhibited a high degree of predictive accuracy for the 10 native species that we modelled (Table 2). The high deviance explained by the model (mean across species = 40%) and high AUC values (mean = 0.77) indicates that the predictive model should be suitable for predicting the native fish species expected to occur at all sampling sites prior to human perturbations.

Overall, we found that the observed native freshwater fish fauna in the Guadiana River basin has experienced homogenization in relation to the reference scenario. When considering only native species, in more than 70% of the possible pairwise comparisons ($n = 12,090$), similarity was higher in the present scenario (i.e. more species are shared among sites) than it had been in the reference situation ($\Delta CS > 0$; Fig. 2). Almost one-fifth of the comparisons (19.5%) showed ΔCS values over 0.5, which indicates an increase in compositional similarity by 50% (50% more species in common), while in 5.5% of comparisons, ΔCS values were over 0.8 (increase in 80%). On the other hand, only 2.3% of comparisons showed a differentiation over 0.5 ($\Delta CS < 0.5$; Fig. 2). Changes in fish faunal similarity were slightly different when the entire observed assemblage (including introduced species) was considered in the analyses. Overall, the extent of differentiation was slightly lower when introduced species were included in the analyses (Fig. 2). The test of multivariate dispersions of compositional similarity supported these results showing that observed native faunas are more similar across the basin than in the reference scenario ($F = 24.98, P < 0.001$). Using the

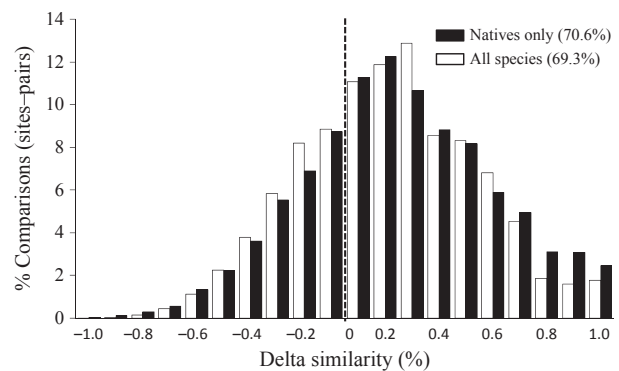


Figure 2 Change in compositional similarity measured as the difference in the Bray–Curtis index of each pair of sites under the current and reference scenarios ($\Delta CS = CS_{current} - CS_{reference}$) for native species only (white bars) and including introduced species (black bars). Positive values indicate increase of compositional similarity or homogenization. The proportion of pairwise comparisons with $\Delta CS > 0$ (homogenization) for the reference and observed scenarios is also shown (upper right corner).

same test, we found significant differences in native compositional similarity along both natural gradients under reference conditions ($F = 4.44, P = 0.004$ and $F = 4.22, P = 0.009$ for $PC1_{nat}$ and $PC2_{nat}$, respectively) and no significant differences in observed assemblages ($F = 1.90, P = 0.11$ and $F = 1.53, P = 0.17$ for $PC1_{nat}$ and $PC2_{nat}$, respectively; Fig. 3). Compositional similarity tended to increase along the climate gradient and decrease along the river size gradient in the reference scenario. These spatial differences were much less pronounced in the observed scenario (Fig. 3).

When testing the role of multiple potential determinants of change in compositional similarity using SEM (Fig. 4), we found that the abundance of introduced species was essential to explain the observed changes. Almost none of the models where the abundance of introduced species was dropped were

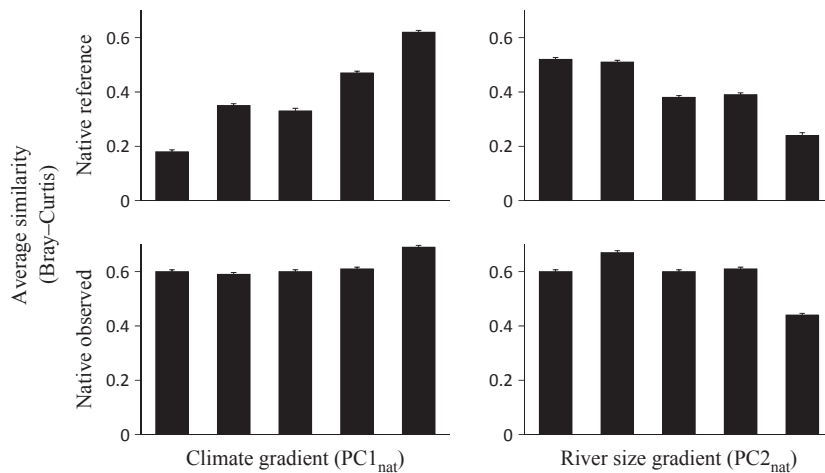


Figure 3 Average compositional similarity (Bray-Curtis) along natural environmental gradients under the reference and observed conditions. Groups were defined using the natural environmental gradient described in Table 1 to contain the same number of sampling sites. Bar errors indicate standard error.

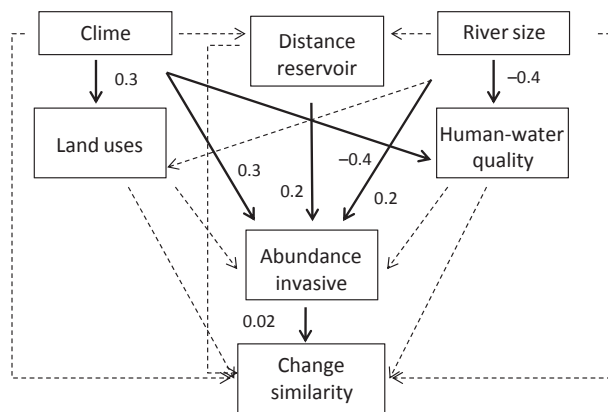


Figure 4 Scheme of structural equation models (SEM) used to test different conceptual pathways of interaction among the change in multivariate dispersions and distance to reservoirs abundance of introduced species. Standardized coefficients based on the correlation matrix for each path are shown. Dotted lines represent non-significant effects.

consistent with our data (significant chi-square in Table 3). In addition, the best model according to AIC was the one that included the abundance of introduced species as the only determinant. The next models in the ranking are different combinations of the abundance of introduced species and other determinants (Table 3). These results are also consistent with the estimates of standardized regression coefficients from the full model, since none of the determinants except the abundance of introduced species had a significant effect on the change of compositional similarity (Fig. 4). Moreover, the distance to reservoirs and the natural gradients were the only variables that had significant effects on the abundance of introduced species, which was higher in areas close to reservoirs and in larger river reaches (Fig. 4).

DISCUSSION

There is a growing agreement among ecologists about the importance that species composition (and not simply species

richness) plays in maintaining ecosystem function and ultimately the maintenance of biodiversity. Rooney *et al.* (2007) called for conservation biologists to consider biodiversity homogenization as an important threat to the conservation of biological diversity. Biodiversity distinctiveness is a character not only worth preserving when attending to criteria such as the maintenance of population genetics or functional ecosystems (Olden *et al.*, 2004, 2010; Rahel, 2010) but also an important attribute used in some of the most common conservation planning strategies (Margules & Pressey, 2000; Ferrier, 2002). Homogenization will make conservation practitioners struggle in the already difficult task of finding the best combination of places that maximize the representation of regional biodiversity while minimizing economic, political or social costs. This will be in detriment to the success of conservation planning and implementation.

One of the key questions when addressing homogenization/differentiation problems from a conservation point of view is how we can best evaluate the process so it reflects changes in biota that are relevant for conservation purposes (Rooney *et al.*, 2007). Here, we have addressed this question from two different points of view. First, we have restricted the analyses to native species for the estimates of the magnitude of change. In this way, we could quantify shifts in compositional dissimilarity and evaluate the implications of these changes for the conservation of native biodiversity more accurately. Although usually done in previous studies (e.g. Falke & Gido, 2006; Olden *et al.*, 2008; Gido *et al.*, 2009), the inclusion of introduced species in the estimates of change in compositional dissimilarity can obscure the underlying homogenization process of native assemblages and the identification of the role that different determinants might be playing. For example, the substitution of native species by introduced ones can lead to maintenance of compositional dissimilarity at the local scale or even led to differentiation processes at broader scales (e.g. catchments) (Rahel, 2010). It may also be unclear whether changes in faunal dissimilarity are because of losses of native species, gains in introduced species or both. Second, we compared the observed compositional dissimilarity with the expected in reference conditions. Given the constraints

Table 3 Summary of the 16 models tested in the structural equation modelling analysis. The chi-square statistic and *P* values measure the probability that the observed and expected covariance matrices differ by more than would be expected because of random sampling errors (Mitchell, 1993). Significant differences stand for models that do not fit the structure of the data being analysed. Models are ranked according to their Akaike information criterion (AIC).

Land uses	Human/water quality	Distance reservoir	Abundance introduced	d.f.	Chi-square	<i>P</i>	AIC
			X	7	8.3	0.31	0.340
		X	X	6	8.0	0.24	0.351
	X		X	6	8.1	0.23	0.352
X			X	6	8.2	0.22	0.353
	X	X	X	5	7.8	0.17	0.363
X		X	X	5	8.0	0.16	0.365
X	X		X	5	8.0	0.15	0.365
X	X	X	X	4	7.7	0.10	0.367
X				7	12.8	0.07	0.370
	X			7	12.9	0.07	0.371
		X		7	13.0	0.07	0.371
X	X			6	12.6	0.05	0.383
X		X		6	12.8	0.04	0.383
	X	X		6	12.8	0.04	0.384
X	X	X		5	12.6	0.02	0.396
				8	75.3	<0.01	0.779

imposed by the lack of knowledge on historical species distributions at fine spatial scales (e.g. river and stream reaches), we used predictive models to estimate probabilities of occurrence of native species in reference (i.e. pre-disturbance) conditions. By comparing observed compositional similarity values against those expected in reference conditions, we accounted for natural spatial patterns of species turnover and were able to better discriminate the determinants of native assemblage homogenization.

Our analyses showed that, in most of the sites examined, compositional similarity of native freshwater fish assemblages in the Guadiana River basin have exhibited a clear homogenization. This pattern was stronger in areas close to reservoirs where introduced species were also more abundant. However, distance to reservoirs did not have significant direct effects on the observed change of compositional dissimilarity, but indirectly through their relationship with introduced species. A similar role of reservoirs as centres of homogenization had been reported before, for both within reservoirs (Clavero & Hermoso, 2011) and in upstream river reaches. Gido *et al.* (2009) found distance to downstream reservoir to be a significant (although weak) predictor of fish faunal homogenization patterns in three river basins in the Great Plains in USA and that the relative position in the stream network (a natural longitudinal upstream–downstream gradient) was the most important predictor of homogenization. The pre-eminent role of this natural gradient could be related to the lack of consideration of natural compositional dissimilarity patterns among sites. Here, we accounted for major natural sources of variation from the analyses (as explained above), so we were able to isolate more accurately the changes in compositional similarity caused by degradation/introduced species and evaluate the role of these non-natural determinants of change. It is

well known that river damming promotes invasions by increasing colonization opportunities for non-indigenous species and by enhancing their subsequent establishment success (Clavero *et al.*, 2004; Havel *et al.*, 2005; Clavero & Garcia-Berthou, 2006; Johnson *et al.*, 2008). The favourable and more stable environment conditions in reservoirs facilitate the establishment of introduced species (Moyle & Light, 1996; Ribeiro *et al.*, 2008), which are a common target of stocking practices (Falke & Gido, 2006; Gido *et al.*, 2009). Propagule pressure is also a major factor predicting the success of invaders in colonizing new ecosystems (Kolar & Lodge, 2000), and reservoirs are stepping stones favouring their dispersion through larger areas in the basin (Havel *et al.*, 2005). In this way, a localized habitat perturbations that could not have more implication on native assemblages beyond the area occupied by the reservoir (see Clavero & Hermoso, 2011, for more details on within reservoir homogenization issues) becomes a more serious problem that affects whole basins.

The important role of introduced species in the decline of native freshwater fish populations in mediterranean freshwater environments is well known. Light & Marchetti (2006) found introduced species to be the main determinants of native freshwater fish diversity loss in Californian basins at the catchment scale. Hermoso *et al.* (2011) also confirmed this prevalent role of introduced species in the Iberian Peninsula at a finer scale within the Guadiana River basin. Some other studies in mediterranean climate areas support the idea that modified habitats continue holding native species in the absence of invasions (Baltz & Moyle, 1993; Moyle, 2002). These findings suggest that mediterranean freshwater fish communities could be resistant or resilient to some forms of habitat perturbation, but be very sensitive to predation or competition interactions with introduced species (Hermoso *et al.*, 2009a).

Our results reveal an additional effect of introduced species as important determinants in the loss of natural patterns in native species turnover or compositional dissimilarity. We show how the patterns in the distinctiveness of fish assemblages along natural gradients (altitude–climate and upstream–downstream) were obscured in the observed scenario. This could be mainly because of the loss of some native species in areas colonized by introduced species, such as areas close to reservoirs or lowland reaches. Although predation and competition between native and introduced species are supposed to be the main mechanisms responsible for the loss of native species in highly invaded freshwater systems, we understand little of the interacting mechanisms that lead to native species loss and homogenization (e.g. is it an abundance-mediated process? Which are the more harmful introduced species for native assemblages?). Further studies on these issues are needed to better understand the process of taxonomic homogenization presented here and improve our capacity to respond to this threat.

Olden *et al.* (2004) emphasized the importance of identifying observed patterns of biotic homogenization and understanding their implications for establishing conservation goals aimed at reducing its future ecological effects. The loss of natural patterns in species turnover has important implications for conservation beyond the obvious reduction in the extent of occurrence of particular species and the consequent increase in their risk of extinction. Species contribute individually and collectively to the functional stability of communities and ecosystems (Hooper *et al.*, 2005; Vileger *et al.*, 2010). Taxonomic homogenization usually results in functional homogenization as well due to the loss of ecological specialist and persistence or appearance of spread generalists (Olden *et al.*, 2004). These homogenized assemblages are simplified versions of the original ones that we do not fully understand. For example, we do not know whether homogenized assemblages can maintain the impoverished native species over the long term or the loss of particular species is especially important. Furthermore, we do not know how homogenization processes in certain areas can imperil the future maintenance of native populations at larger (regional and global) scales, so further research is needed. This is especially concerning in the Guadiana River basin where homogenization affects predominantly the areas that formerly supported the most diverse assemblages in the basin. The implementation of efficient conservation for the maintenance of native fish diversity is seriously threatened by the homogenization processes. The identification of priority areas for the conservation is hindered by the fact that the most diverse communities are diminishing, which requires the inclusion of more areas to adequately protect all the species (under reference conditions, it was easier to find a set of areas that complemented each other to represent all the species). Given the predominant role that introduced species play in the homogenization process and their relation with reservoirs, particular attention must be paid to mitigating or preventing these threats.

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SUPPORTING INFORMATION

Additional Supporting information may be found in the online version of this article:

Table S1 Complete list of environmental variables used to characterize habitat in the Guadiana River basin.

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BIOSKETCHES

Virgilio Hermoso is a postdoctoral Research Fellow at the Australian Rivers Institute (Griffith University, Australia). His research interest focuses on the study of threats to the conservation of freshwater biodiversity, especially on the interactive effects of habitat degradation and introduced species, as a way to better inform conservation decision-making.

Miguel Clavero is a Research Fellow at the Donana Biological Station (CSIC, Spain). He is interested in the influences of environmental gradients on the ecology of populations and communities in mediterranean ecosystems, including invasive species and their impacts on biodiversity. He has worked with freshwater fish, birds and semi-aquatic mammals.

Mark Kennard is a Senior Research Fellow at the Australian Rivers Institute (Griffith University, Australia). His research interests include the ecology of freshwater fish, environmental flow management, river bioassessment and conservation planning for freshwater biodiversity.

Author contributions: All the authors conceived the idea and contributed to the writing of the manuscript, which was led by V.H.; V.H. collated and analysed the data.

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